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Amniotic fluid (AF) was collected from 54 women with preterm labor at the Tripler and Madigan Army Medical Centers from 19 Dec 94-30 Sep 95. The AF was evaluated by standard culturing methods for aerobic and anaerobic bacteria as well as Mycoplasma. At the time of amniocentesis, 5 mL of AF was aspirated and frozen for detection of bacteria and Mycoplasma by the polymerase chain reaction (PCR) and for interleukin-6 studies. Approximately 55% of the patient AF samples were positive for bacteria by the PCR, whereas the culture data detected bacteria in approximately 9% of the samples. Interleukin-6 was found to be elevated above 600 pg/mL in nine samples. Patients whose amniotic fluid IL-6 levels exceeded 600 pg/mL delivered preterm. The sensitivity of the PCR assay and the prognostic value of IL-6 as markers of preterm labor and delivery are discussed.

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INTRODUCTION

BACKGROUND:

Idiopathic preterm labor is the approximate cause of one-third of all preterm births (1). Preterm birth is the largest contributor to neonatal, perinatal, and infant mortality in the United States (2). Preterm birth also is a major cause of infant morbidity such as developmental delay, cerebral palsy, and chronic lung disease (3). Several published reports have identified the gravid active duty soldier as at high risk for preterm labor and delivery (4-6). In the multicenter study by Adams, the rate of preterm delivery of white enlisted women was 10.5%. This is higher than the national average rate for preterm birth of 8.5% (7). This same study noted that black enlisted women have a 31% higher risk than white enlisted women for preterm delivery; black enlisted pregnancies had a preterm birth rate of 13.5%. It is important to note that whereas this is higher than the rate in white enlisted pregnancies, it is still lower than the national average for black women of 18.3% (7). These high rates of preterm delivery occur despite free access to prenatal care, a general healthy population, and a low use of recreational drugs (8,9). To date, no data is published concerning the impact of preterm labor and delivery on duty days lost. However, the primary treatment of preterm labor is bedrest; therefore, it can be expected that a significant number of duty days are lost due to this pregnancy complication. Therefore, any regime that would investigate the cause of preterm labor and delivery would be of great benefit not only to the gravid soldier and their families but also for their unit.

Intra-amniotic infection is thought to play a key role in preterm labor. Slightly greater than 9% percent of women with preterm labor and intact membranes will have amniotic fluid cultures positive for bacteria or *Mycoplasma* species (1,10,11). A proposed mechanism in which bacterial infection leads to premature delivery involves activation of cell-mediated immunity. This immune activation process stimulates production of cytokines. This in turn results in prostaglandin production that leads to uterine contractions (12). One of the cytokines that is stimulated in this cascade event is interleukin-6 (IL-6). Several studies have shown that elevated amniotic fluid IL-6 levels from pregnancies complicated by preterm labor are at high risk for preterm delivery (12-17). Positive amniotic fluid cultures also correlate well with elevated IL-6 levels. However, over 50% of pregnancies that have elevated IL-6 levels have negative amniotic fluid cultures (12). It is unknown if this is due to the poor sensitivity of the culture methods to detect bacteria or if there is another etiology for the stimulation of cytokine production.

Since bacterial infection of the amniotic fluid has been suggested as a cause of preterm labor, several studies have employed antibiotics as part of the treatment for preterm labor. The results of these trials in prolonging pregnancy have been mixed and no clear cut benefit has been proven for the use of antibiotics in preterm labor (18). This may be due to the inability to select the patient population that would best benefit from antibiotic therapy, i.e. those with intra-amniotic infection. It is possible that better results could be obtained by using a very sensitive technique such as the polymerase chain reaction (PCR) to detect the presence of bacteria in the amniotic fluid and treat those patients with antibiotics. Cases in which the IL-6 levels are elevated

but no bacterial DNA is present may represent a non-infective immune
process which would require the development of new treatment strategies,
such as immunosuppression.

STATUS:

In this study, we had originally proposed enrolling a minimum of 150
pregnant women from Tripler (TAMC) and Madigan (MAMC) Army Medical
Centers. However, due to the late arrival of funds (mid-December 1994),
an overestimate of the patient population, and the end of the fiscal
year (only nine months for the study), we enrolled 64 women. Of these 64
women, ten were excluded from data analysis due to the induction of
delivery due to preeclampsia, the presence of twins, or upon clinical
reevaluation, failure to meet inclusion criteria. Amniotic fluid samples
(n = 54) were collected from these pregnancies complicated by preterm
labor and were obtained by consent in clinically indicated situations
that required analysis to rule out chorioamnionitis. At the time of
amniocentesis the fluid was gram-stained and cultured for aerobic and
anaerobic bacteria as well as *Mycoplasma*. Amniotic fluid was evaluated
for the presence of bacteria and *Mycoplasma* utilizing the polymerase
chain reaction (PCR) to amplify a ribosomal concensus sequence of DNA.
The presence of such sequences in amniotic fluid was considered as
evidence of bacterial contamination. Interleukin-6 (IL-6) levels were
obtained as well and were correlated with clinical outcomes and the PCR.
After delivery, placentas were sent to pathology for observation of
polymorphonuclear cells in formalin-fixed, paraffin-embedded chorion or
amnion. The presence of polymorphonuclear cells was evidence for
chorioamnionitis.

MATERIALS & METHODS

PATIENT ENROLLMENT CRITERIA:

The study population consisted of women 18 years or older admitted with the diagnosis of preterm labor (regular uterine contractions with a change in cervical dilation between 20-37 weeks) or advance cervical dilation that required amniocentesis to rule out intrauterine infection. Patients with intact membranes and those with premature rupture of the membranes were included. Prior treatment with antibiotics was not an exclusionary factor.

COLLECTION OF AMNIOTIC FLUID & BACTERIAL DNA ISOLATION:

At the time of amniocentesis, the amniotic fluid was collected aseptically and an aliquot removed for clinical analysis. Clinical analyses included the aerobic and anaerobic bacterial cultures, glucose determinations, and gram stains. *Mycoplasma* culturing for TAMC and MAMC was done at MetPath Laboratories in Teteroboro, New Jersey.

Additional fluid was collected by aspiration and transfered to a sterile container for PCR and IL-6 studies. This fluid was briefly spun at 1,000 x g, split into two equal fractions, and frozen. The supernatant phase was designated specifically for the IL-6 ELISA in order to minimize the contribution from intracellular IL-6 from leukocytes upon freezing. Samples collected at the TAMC were processed and shipped frozen on dry ice to the Department of Clinical Investigation (DCI) at MAMC for further analyses for IL-6 and PCR studies. Samples from MAMC were generally processed within 24 hours after amniocentesis at the DCI.

The bacterial DNA was isolated from clinical samples by the method of Goldenberger *et al.* using SDS and proteinase K (19). Of seven bacterial DNA isolation methods evaluated, it was determined to be the best and most effective for gram (+) and gram (-) organisms. Essentially, the amniotic fluid was frozen overnight at -20°C, quickly thawed at 37°C, and subjected to centrifugation at 12,000 x g for 10 minutes at 4°C. The fluid above the pellet was carefully aspirated and discarded. The pellet was resuspend with 0.2 mL of the digestion buffer (50 mM Tris-HCl, 1 mM EDTA, and 0.5% SDS) with 200 µg/mL of proteinase K and placed in a shaking incubator at 55°C for three hours with vigorous agitation (240 RPMs).

After the proteinase K digestion, the enzyme was heat inactivated at 95°C for 10 minutes, the sample cooled to 4°C, and centrifuged at 12,000 x g for 10 minutes at 4°C. The supernatant was transfered to another sterile tube and and stored at -20°C until needed. Approximately 10 µL of the supernatant was sufficient for a 100 µL PCR.

POLYMERASE CHAIN REACTION:

PCR was performed according to standard methodology with one modification (20,21). Tween-20 (Sigma) was included in the final PCR at a concentration of 2% (19). This was reported to prevent inhibition of *AmpliTaq* polymerase® by minute concentrations of residual SDS from crude DNA extractions. In addition, digoxigenin-11-dUTP (Boehringer Mannheim, Inc.) was directly incorporated into the PCR product to

facilitate detection by chemiluminescence and to minimize false negative
PCR results (22).

EUBACTERIAL PCR

Consensus eubacterial primers to the 16S rRNA were synthesized
commercially (Gibco BRL). The sequences were as follows:

16S rRNA Eubacterial Primers (23)
Upstream Primer: 5'-AGA GTT TGA TCC TGG CTC AG-3'
Downstream Primer: 5'-CCG TCA ATT CCT TTG AGT TT-3'

The cycling parameters were 94°C for 30 seconds, 56°C for 30 seconds,
and 72°C for 45 seconds for 30 cycles in a Perkin-Elmer 9600
Thermocycler. The resulting PCR product was 900 bp in length.

MYCOPLASMA PCR

Primers for *Mycoplasma* were genus-specific and were synthesized on
an oligo-DNA synthesizer, Gene Assembler Special (Pharmacia LKB). Primer
sequences were as follows:

16S rRNA Genus-Specific Primers (24)
Upstream Primer: 5'-ACT CCT ACG GGA GGC AGC AGT A-3'
Downstream Primer: 5'-TGC ACC ATC TGT CAC TCT GTT AAC CTC-3'

The cycling parameters were 94°C for 30 seconds, 60°C for 30 seconds,
72°C for 60 seconds for 30 cycles. The resulting PCR product was 717 bp
in length.

Amniotic fluid samples that were PCR positive for *Mycoplasma* were
assigned to the PCR positive group and further evaluated with species-
specific DNA primers for *Mycoplasma genitalium* and *Ureaplasma
urealyticum*. Primer sequences were as follows:

Mycoplasma genitalium ATT Primers (25,26)
Upstream Primer: 5'-TGT CTA TGA CCA GTA TGT AC-3'
Downstream Primer: 5'-CTG CTT TGG TCA AGA CAT CA-3'

Ureaplasma urealyticum Urease Primers (27)
Upstream Primer: 5'-CCA GGA AAA CTA GTA CCA GGA GC-3'
Downstrean Primer: 5'-CTC CTA ATC TAA CGC TAT CAC C-3'

The thermocycling parameters were as described for the *Mycoplasma*
consensus primers. The resulting PCR products for *Mycoplasma genitalium*
and *Ureaplasma urealyticum* were 300 bp and 460 bp, respectively.

SOUTHERN BLOTTING & CHEMILUMINESCENCE:

The PCR products were size-fractionated by electrophoresis on 2%
agarose gels with 0.5 µg/mL ethidium bromide in 1X TBE (0.089 M Tris-
borate, 0.089 M boric acid, and 0.002 M EDTA) buffer (28). After
photography of the gel by UV irradiation on a Foto Eclipse (Fotodyne),
the DNA was transferred to nylon membranes (Managraph, Micron
Separations, Inc.) by the method of Southern (29). Upon completion of
the transfer, the membrane was briefly immersed in 2X SSC (0.3 M sodium
chloride and 0.03 M sodium citrate), and the nucleic acids crosslinked
by UV irradiation at 120,000 µJ (Stratalinker, Stratagene). The

membranes were processed for chemiluminescence as described (22) and in
accordance with the manufacturer's recommendations (Boehringer Mannheim,
Inc.). Typical luminograph exposures with XAR-5 film (Kodak) were one,
five, and thirty minutes.

INTERLEUKIN-6 ASSAYS

Frozen amniotic fluid was assayed quantitatively for interleukin-6
(IL-6) levels with the Human ELISA IL-6 kit (Endogen, Inc.) in
accordance with the manufacturer's recommendations. Amniotic fluid
samples that were difficult to quantitate due to concentration were
diluted ten-fold with the kit standard diluent. Even with a 1:10
dilution patient, T052's IL-6 level was significantly greater than the
upper confidence limits of the assay. Values were reported as pg/mL.

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RESULTS

PATIENT ENROLLMENT & CLINICAL DATA:

A total of 54 patients were finally included in the study. Table I
summarizes the demographic data. The clinical profile of the study
population is described (Table II). Aproximately 67% of the patients (n
= 36) delivered preterm. The mean gestational ages at the time of
amniocentesis and at the time of delivery were 32.5 weeks and 35.6
weeks, respectively. Table III lists the organisms identified in the
amniotic fluid cultures. The overall culture positive rate was 9.2% (n =
5). A summary of the patient population at each institution follows.

Tripler Army Medical Center

A total of 55 amnioceteses were performed and all patients
delivered at TAMC. Subsequently, nine were excluded from further
analysis due to twinning (n = 7), induction of delivery due to
preeclampsia (n = 1), or delivery less than 20 weeks (n = 1). Enrollment
of patients began in January for an average of six patients per month.

Fifty percent of the TAMC patients enrolled delivered preterm with
43% of the newborn weights less than 2,500 grams. This was consistent
with previous studies of patients that required amniocentesis to rule
out infection (1). Of the amniotic fluid cultures, five were positive
for bacteria and/or *Mycoplasma* (2 aerobic, 1 anaerobic, and 2
Mycoplasma) (Table III). Patient T005 was culture positive for a
Lactobacillus spp. and a *Neissria spp.*, patient T019 was positive for
Ureaplasma urealyticum (T-strain), patient T032 was positive for a
coagulase negative *Staphylococcus,* T049 was positive for
Propionibacterium acnes, and patient T052 was positive for *Ureaplasma
urealyticum* (T-strain) and a *Fusobacterium spp*. The cultures for
patients T032 and T019 were considered skin flora contamination. Nine
patients have been diagnosed with clinical chorioamnionitis and nine
pathological diagnoses of chorioamnionitis have been noted on
examination of placentas.

Madigan Army Medical Center

A total of eleven amnioceteses were performed and eleven patients
delivered at MAMC. One patient was subsequently found not to meet the
inclusion criteria and was excluded from data analysis. A second was
excluded due to twins.

Fifty percent of the patients enrolled delivered preterm with 20% of
the newborn weights less than 2,500 grams. The average birth weight
was 2,910 grams. Of the nine amniotic fluid cultures, none were positive
for bacteria (aerobic, anaerobic, or *Mycoplasma*). One patient was
diagnosed with clinical chorioamnionitis with the same pathological
results given. No other patients were diagnosed with clinical
chorioamnionitis. Only two other placentas were sent for pathological
evaluation and one of these was found to have evidence of acute
chorioamnionitis despite no evidence clinically. Administrative errors
resulted in the failure to send other placentas for pathological
examination.

No complications from the amniocentesis procedures at TAMC and MAMC
were noted.

POLYMERASE CHAIN REACTION DATA:

EUBACTERIAL PCR DATA

The optimization of the bacterial PCR studies included ascertaining
the most optimal Mg^{2+} concentrations (Figure 1). Briefly, *Escherichia
coli* DNA was amplified with the 16S rRNA concensus primer set at 0.5 mM
increments of $MgCl_2$ from 1.5 mM-4.0 mM. The most optimal Mg^{2+}
concentration was found to be 2 mM, whereas Mg^{2+} levels in excess of 3
mM resulted in either reduced or complete inhibition of signal on
ethidium bromide-stained agarose gels. Although there was an absence of
the 900 bp PCR product at 3.5 mM of $MgCl_2$ on the agarose gel, it was
detectable on the luminograph by chemiluminescence.

Upon completion of standardization of the PCR conditions, the 16S
rRNA concensus primer sets were evaluated against a number of bacterial
organisms (Figure 2). Bacterial DNA was isolated from *Enterobacter
aerogenes*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*,
Streptococcus lactis Serratia marcescens, and Group B *Streptococcus*
(GBS) (gifts of Dr. Robert S. Stewart, Jr., Stephen F. Austin State
University, Nacogdoches, TX). The results were uniform and consistent
with earlier observations. All bacterial organisms evaluated by the PCR
resulted in a 900 bp product. Although no PCR product was observed in
the GBS lane, it was readily apparent on the luminograph (data not
shown). No cross-reactive PCR products were observed with human genomic
lymphocytic DNA on the agarose gel or luminograph.

Amniotic fluid from pregnancies greater than 22 weeks is primarily
composed of fetal urine. Amorphorous salts in urine were reported as
potent PCR inhibitors that required additional measures to counteract.
Furthermore, the bacterial DNA recovery method generates a crude extract
that could contain potential inhibitors of the PCR. In order to address
these concerns, control, non-preterm amniotic fluid (>22 weeks
gestation) from the MAMC Department of Pathology was obtained. It was
spiked with different concentrations of either GBS or *E. coli*. The
samples were processed as described in the methods section. Nineteen
microliters of the lysate was added to the PCR with 1 ng of lambda DNA.
The results of the subsequent PCR are presented in Figure 3. In all
samples, the lambda DNA was amplified and a 500 bp product was generated
as seen by ethidium bromide-staining on agarose gels.

The sensitivity of the reaction also was evaluated (Figure 4).
Amniotic fluid was spiked with either GBS [gram (+)] or *E. coli* [gram (-
)] at dilutions of 10^7-10^2 organisms/mL. The PCR products were obtained
from both organisms at all concentrations. This was consistent with
other observations that the sensitivity of the PCR may approach one to
ten microorganisms per biological fluid sample (30,31). Interestingly,
PCR products were observed in the unspiked amniotic fluid control lane.
The non-preterm control amniotic fluid was an excess clinical specimen
obtained from the Department of Pathology. The history of the fluid was
unknown so the potential exists that it was contaminated with bacteria
prior to the initiation of our studies. This would explain the faint
presence of the 900 bp band in the AF lane. We had previously

demonstrated that the primer sets were not cross-reactive with human DNA
(Figure 2).

The utility of these primers for the PCR diagnosis of bacterial
infections in amniotic fluid is demonstrated in Figure 5. Amniotic fluid
from TAMC patients T005, T006, and T007 were processed as described and
subjected to 30 cycles of the PCR. The ethidium bromide stained agarose
gel is presented. Patients T005 and T006 are clearly PCR positive
whereas patient T007 is PCR negative. The luminograph data is in
agreement with these findings (data not shown). The bacterial culture
data for patients T005 and T007 were positive and negative,
respectively. This is in agreement with the PCR data. However, T006 was
bacterial culture negative but PCR positive.

Of the 54 amniotic fluid samples, approximately 56% (n = 30) were
positive for bacteria by the PCR. No demographic or clinical differences
were observed between the bacterial PCR positive and negative patients
(Table IV). The PCR positive group had a shorter time from amniocentesis
to delivery (TFATD) interval (μ difference = 12.6 days, p = 0.02) and
lower newborn weights (μ difference = 420 grams, p = 0.02). Whereas
gestational age tended to be less in the PCR group than a week, this was
not   statistically significant (p = 0.48). The only death occurred in
the PCR positive group. The infant was born at 24.4 weeks gestation, and
the cause of death was attributed to extreme prematurity.

MYCOPLASMA PCR DATA

The processed amniotic fluid samples were evaluated with the
consensus 16S rRNA *Mycoplasma* primers. Whereas none of the MAMC patients
were culture or PCR positive for *Mycoplasma*, two TAMC patients were
culture and PCR positive. Interestingly, only one of the culture
positive patients, T052, was observed to be PCR positive (Figure 6).
Patient T019 was found to be culture positive for *Ureaplasma
urealyticum*; however, she was *Mycoplasma* negative by the PCR. Due to a
clerical shipping error, no culture data for T006 was available, but she
was determined to be PCR positive.

Patients T052 and T006 were assigned to the PCR *Mycoplasma* positive
group. Additional studies with the DNA primers for *Mycoplasma genitalium*
and *Ureaplasma urealyticum* were negative for patient T006 (Figure 7).
Whereas patient T052 was negative for *Mycoplasma genitalium*, she was PCR
positive for *Ureaplasma urealyticum* which was in agreement with the
Mycoplasma culture data.

INTERLEUKIN-6 ELISA

IL-6 concentrations greater than 600 pg/mL were considered to be
elevated and evidence of a positive result. Applying this threshold, 17%
of the samples were positive and all of the positive patients (n = 9)
delivered preterm. A summary of the pregnancy and newborn outcomes for
the IL-6 groups is presented (Table VI). The positive IL-6 group
delivered on average 5.2 weeks earlier and weighed 1,071 grams less than
term deliveries (p<0.0001). The length of the newborn stay was greater
in the IL-6 positive group with the positive group staying on average
20.6 more days (p<0.0001). Table VII lists the elevated IL-6 patients
along with their clinical outcomes and PCR data (bacteria and
Mycoplasma). Of the nine elevated IL-6 amniotic fluid samples, six (66%)
were positive for bacteria by the PCR. One PCR patient with elevated IL-

6 was also PCR positive for *Mycoplasma*. Three of the elevated IL-6
patient samples were negative for *Mycoplasma* and bacteria by culture and
PCR. Due to a lack of compliance with the protocol, insufficent numbers
of placentas were collected to perform an adequate analysis of
histologic evidence of chorioamnionitis.

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CONCLUSIONS

In order to perform a prospective investigation concerning the role
of infection in preterm delivery a study population with a high
incidence of preterm delivery was required. With a preterm delivery rate
of 67% in our study population, our target population was appropriate.
The incidence of positive amniotic fluid cultures is consistent with
other published reports in this pregnant population (1).

One of the objectives of this study was to determine the feasibility
of using the PCR to detect evidence of a prior or present infection in
amniotic fluid. The utility of this approach for diagnosis of intra-
uterine infections has been previously illustrated for *Toxoplasma gondi*
(32,33) and *Treponema pallidum* (30,34).

Although great precautions were taken to eliminate bacterial
contamination from skin flora and other sites, we were concerned that
all samples may be positive due to the sensitivity of the technique. The
fact that 40% of the samples returned with no evidence of bacterial
infection proves that this technique can be employed and extraneous
bacteria avoided.

Another objective was to determine the incidence of bacterial
contamination in amniotic fluid detected using the PCR technique. As
suspected, the prevalence of the PCR evidence of bacterial contamination
in amniotic fluid was much greater than the bacterial culture data. The
finding of 55% of pregnancies presenting with preterm labor having
evidence of bacteria by the PCR strongly suggests an association between
preterm labor and infection. Furthermore, pregnancies with evidence of
bacterial contamination by the PCR had a shorter time from amniocentesis
to delivery interval and had lower birth weights. Although gestational
length was shorter in the PCR positive group by a week, this was not
statistically significant. We suspect that many of the PCR positive
samples were secondary to skin contamination. Although bacterial
speciation is possible using the PCR and DNA hybridizations, it was
beyond the scope of this investigation due to the time and funding
constraints. It is likely that the use of typing would help determine
which positive samples were due to skin contamination and which were
true pathogens in the amniotic fluid. In addition, further investigation
is needed to determine the incidence of positive PCR samples in a low
risk population such as term, uncomplicated deliveries.

IL-6 proved to be a very sensitive and prognostic marker for
pregnancies that were destined to deliver preterm. All of the elevated
IL-6 samples delivered preterm. The high IL-6 groups delivered earlier,
weighed less, and had longer newborn stays. This is consistent with the
findings of several other investigations (17).

Our last objective was to determine the incidence of bacterial
contamination in amniotic fluid samples with elevated IL-6
concentrations. Previous studies using culture techniques noted that
about 50% of these samples were culture positive (13). Our objective was
to determine if a more sensitive technique, such as PCR would find a
higher incidence of bacterial presence in this population since IL-6 is
thought to be elevated in response to infection. Of the nine samples
that were elevated 66% were positive for bacteria.

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Of interest are the three amniotic fluid samples that were culture
and PCR negative but had elevated IL-6 levels. Two possible causes exist
to explain a 33% negative predictive value (although more may exist).
Infection limited to the decidual fetal membrane interface could
stimulate the release of cytokines from inflammatory cells, which cross
into the amniotic fluid. It is conceivable that IL-6 is being stimulated
by another factor, such as a noninfective immune process.

Clinically, those patients with elevated IL-6 and evidence of
bacterial infection may benefit from aggressive treatment with
tocolysis. On the other hand, there may be a role for those patients
with no evidence of bacterial infection to receive immunosuppressive
therapy. Additional studies to resolve these issues await further
investigation.

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MWM

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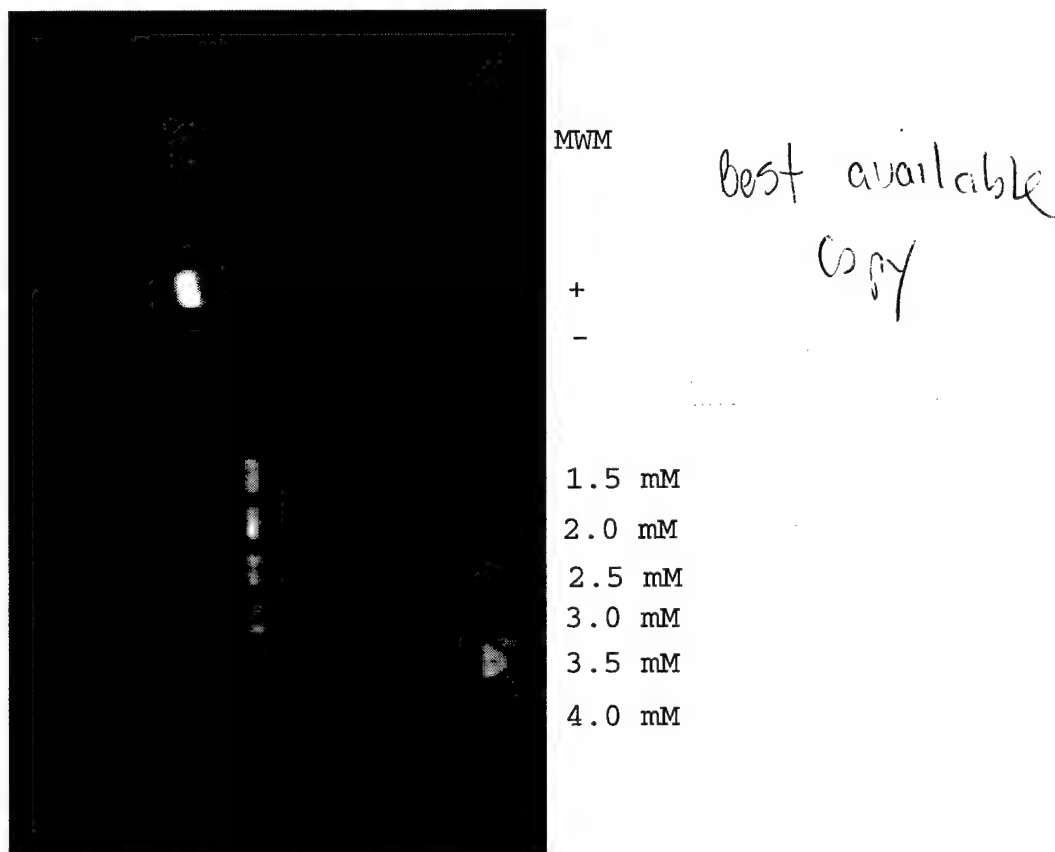copy

1.5 mM
2.0 mM
2.5 mM
3.0 mM
3.5 mM
4.0 mM

Figure 1

Figure 1. Titration of $MgCl_2$ for the 16S rRNA eubacterial primers. The
PCR was performed as described with *Escherichia coli* DNA. MWM is the
digoxigenin-labeled molecular weight marker VIII (Boehringer Mannheim,
Inc.), (+) is the lambda DNA control PCR positive (500 bp), and (-) is
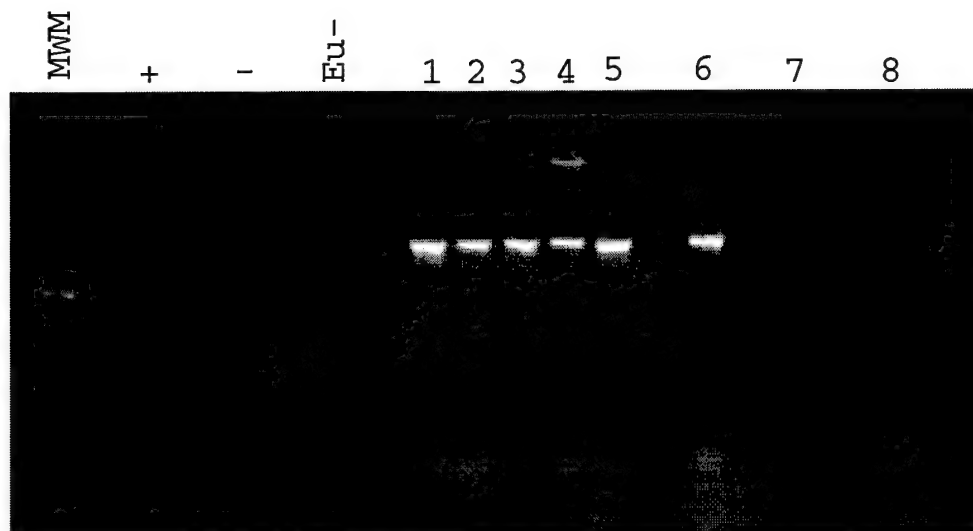the lambda control PCR negative.

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Figure 2

Figure 2. Evaluation of eubacterial 16S rRNA concensus primers against
cultured bacterial pathogens. MWM, digoxigenin-labeled molecular weight
marker VIII (Boehringer Mannheim, Inc.); (+), lambda DNA control PCR
positive (500 bp); (-), lambda control PCR negative; Eu(-), eubacterial
control PCR negative; 1, *Enterobacter aerogenes*; 2, *Escherichia coli*;
3, *Bacillus subtilis*; 4, *Staphylococcus aureus*; 5, *Streptococcus lactis*;
6, *Serratia marcescens*; 7, human genomic DNA; and 8, 10^5 Group B
Streptococcus bacteria.
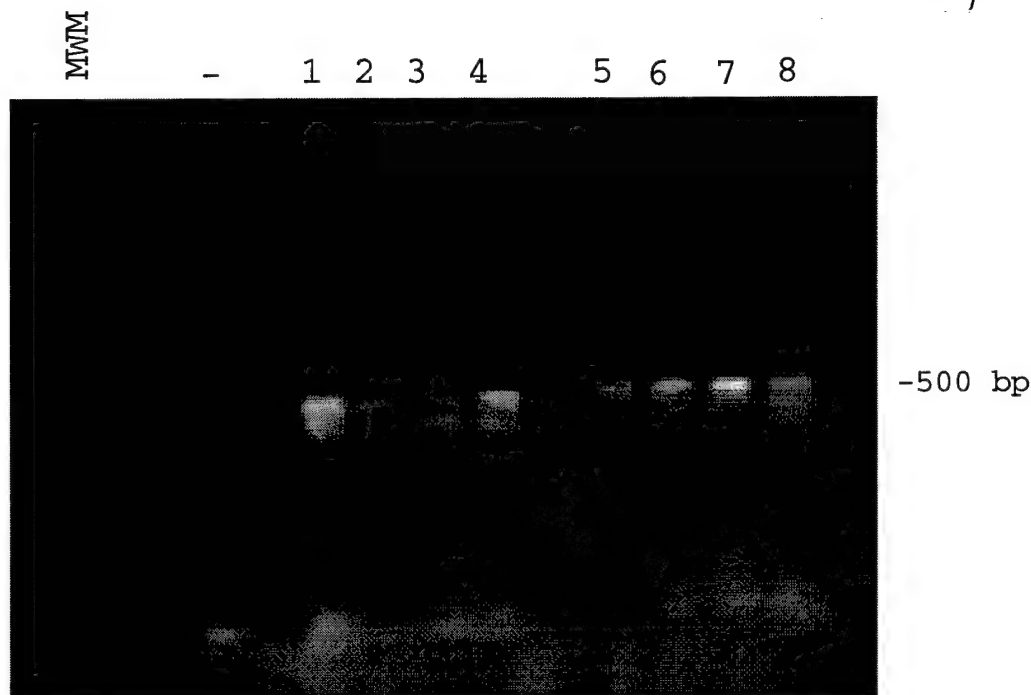
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Figure 3

Figure 3. PCR of lambda DNA from amniotic fluid spiked with Group B *Streptococcus* (GBS) and *E. coli*. One mL of control amniotic fluid was spiked with various concentrations of bacteria. Lambda DNA (1 ng) was added to 19 μL of the lysate and subjected to PCR. Lanes: MWM, digoxigenin-labeled molecular weight marker VIII (Boehringer Mannheim); (-), lambda control negative; 1, 10^8 GBS; 2, 10^6 GBS; 3, 10^4 GBS; 4, 10^2 GBS; 5, 10^8 *E. coli*; 6, 10^6 *E. coli*; 7, 10^4 *E. coli*; and 8, 10^2 *E. coli*.
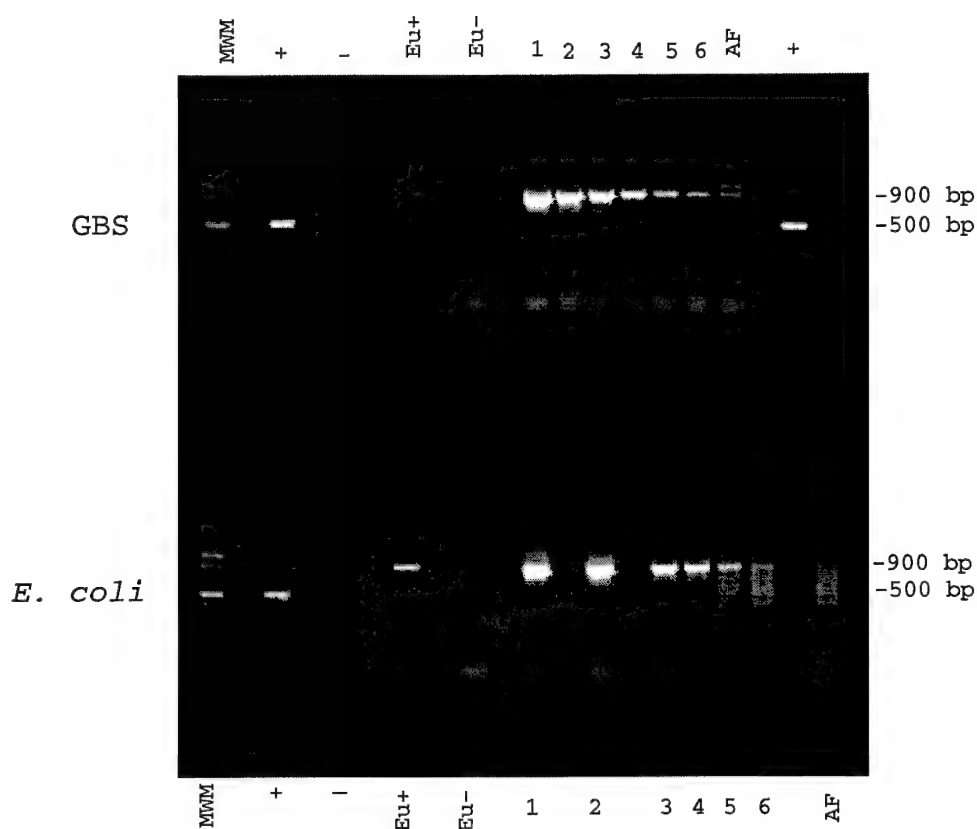
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Figure 4

Figure 4. Sensitivity of eubacterial 16S rRNA primers. One mL of control
amniotic fluid was spiked with Group B *Streptococcus* and *E. coli* at
various concentrations. Bacterial DNA was recovered and the PCR was as
described in the Materials & Methods. Lanes: MWM, digoxigenin-labeled
molecular weight marker VIII (Boehringer Mannheim, Inc.); (+), lambda
DNA control PCR positive, 500 bp; (-), lambda control PCR negative;
Eu(+), *Streptococcus lactis* control PCR positive, 900 bp; Eu(-),
eubacterial control PCR negative; 1, 10^7 bacteria; 2, 10^6 bacteria; 3,
10^5 bacteria; 4, 10^4 bacteria; 5, 10^3 bacteria; 6, 10^2 bacteria; and AF,
unspiked amniotic fluid.
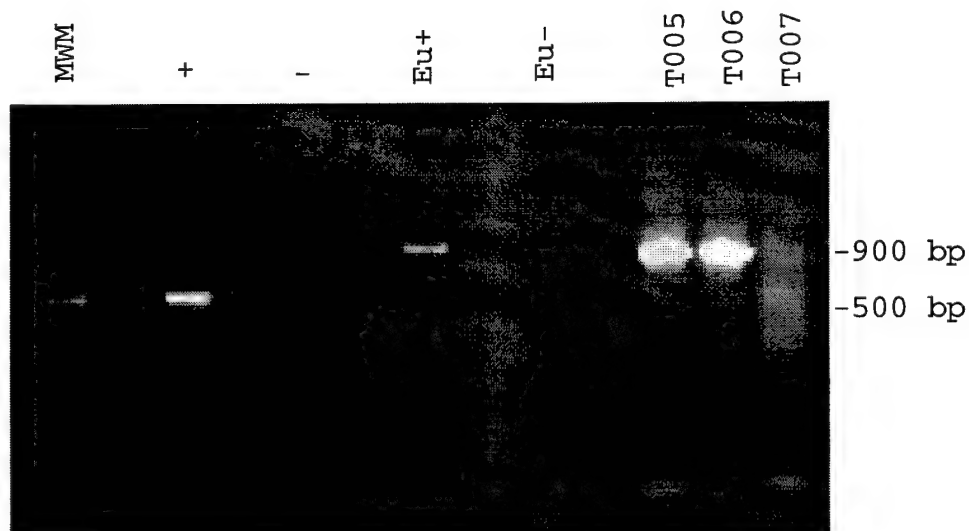
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Figure 5

Figure 5. Eubacterial PCR of patient amniotic fluid samples. Lanes: MWM,
digoxigenin-labeled molecular weight marker VIII (Boehringer Mannheim,
Inc.); (+), lambda DNA control PCR positive, 500 bp; (-), lambda control
PCR negative; Eu(+), *Streptococcus lactis* positive control, 900 bp; EU(-
), eubacterial control PCR negative; and T005-T007, patients' amniotic
fluid samples. Patients' T005 and T006 are PCR positive for bacteria.
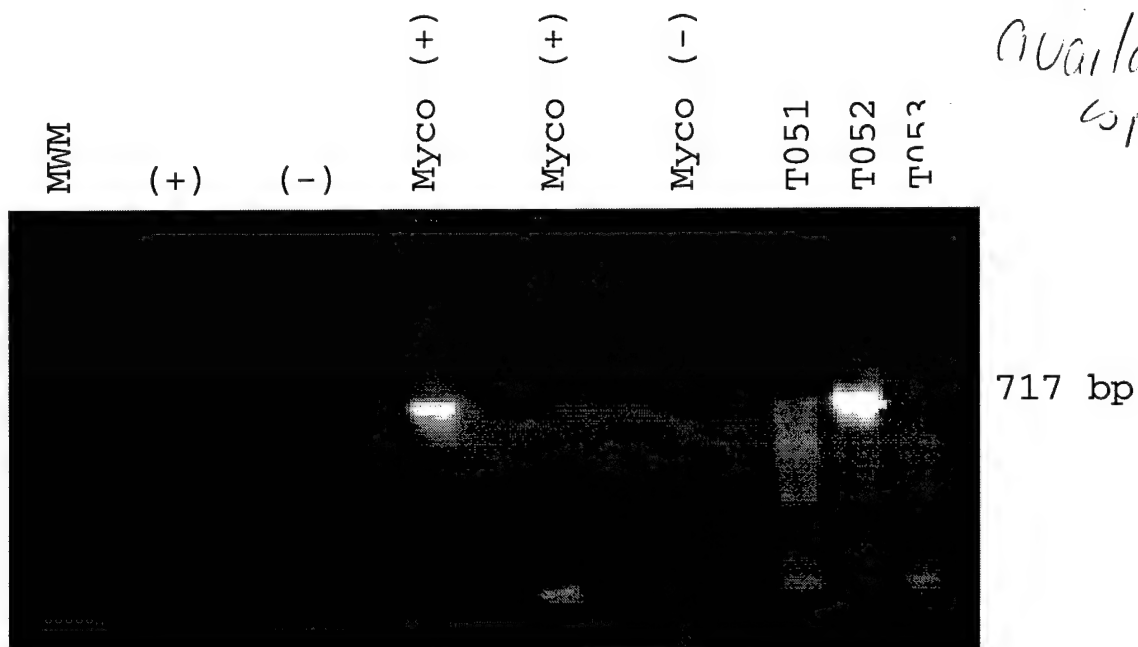
Figure 6. *Mycoplasma* PCR of patient amniotic fluid samples with
Mycoplasma concensus 16S rRNA primers. Lanes: MWM, digoxigenin-labeled
molecular weight marker VIII (Boehringer Mannheim, Inc.); (+), lambda
DNA control PCR positive, 500 bp; (-), lambda control PCR negative; Myco
(+), *Mycoplasma* control PCR positive; Myco (-), *Mycoplasma* control PCR
negative; and T051-053 patient amniotic fluid patient samples. Patient
T052 is PCR positive.

21
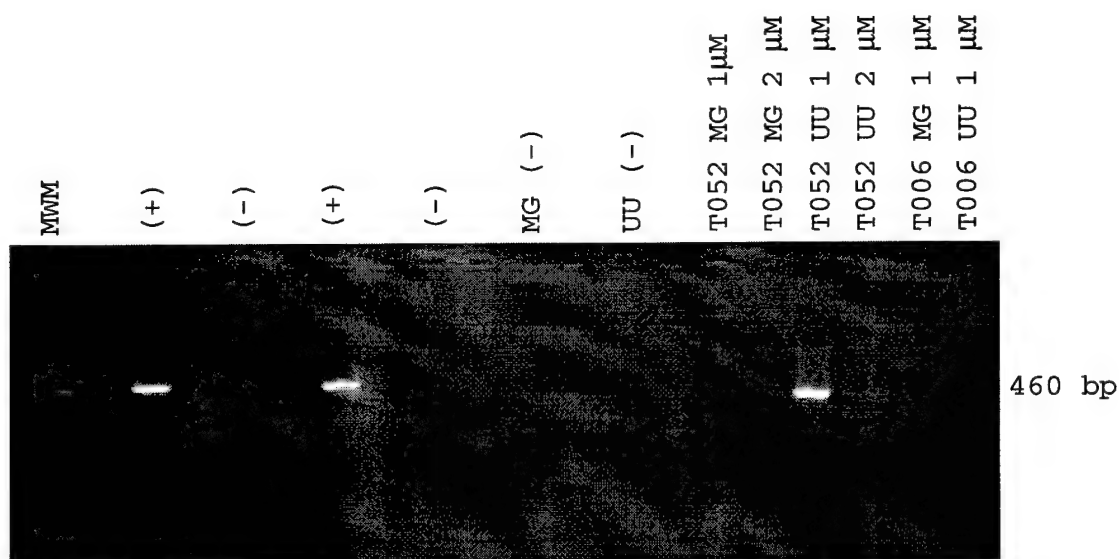
Figure 7. *Mycoplasma* PCR positive patient amniotic fluid samples (T052
and T005) evaluated with species specific primers. Lanes: MWM,
digoxigenin-labeled molecular weight marker VIII (Boehringer Mannheim,
Inc.); (+), lambda DNA control PCR positive, 500 bp; (-), lambda control
PCR negative; MG (-), *Mycoplasma genitalium* control PCR negative; UU (-
), *Ureaplasma urealyticum* control PCR negative; and T052-006 patient
amniotic fluid samples with *Mycoplasma genitalium* and *Ureaplasma
urealyticum* primers at 1 and 2 μM concentrations. Patient T052 is
clearly PCR positive for *Ureaplasma urealyticum* at 1 μM of primers.

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Table I
Patient Demographics

- **Mean Maternal Age**   **25.3 years**
- **Nulliparous Patients**   **27 (50%)**
- **Race**

White	**28 (52%)**
Black	**12 (22%)**
Other	**14 (26%)**

Table II
Clinical Characteristics

- **Number of Patients**   54
- **Preterm Deliveries (< 37 Weeks)** 36 (67%)
- **Mean Age at Delivery**   35.6 wks (3.1)*
- **Mean Age at Amniocentesis**   32.5 wks (3.1)*
- **Mean Newborn Weight**   2,792 g (670)*
- **Mean Newborn Hosp. Stay**   7.3 days (1.2)*
- **Mean Cervical Dilation**   2.0 cm (1.2)*

*= standard deviation

Table III
Amniotic Fluid Positive Cultures

Patient No.	Organism	EGA	Complications
T005	*Lactobacillus* spp. *Neisseria* spp.	33 wk	Congenital Pneumonia
T019	*Urea urealyticum*	34.1 wk	None
T032	Coagulase-negative *Staphylococcus*	35.9 wk	None
T049	*Propionibacterium acnes*	36.6 wk	None
T052	*Fusoform* spp.	27.6 wk	Suspect Sepsis

EGA = estimated gestational age at amniocentesis

Table IV
Demographics & Clinical Data by PCR Status

	(+) (n = 30)	(-) (n = 24)	p-Value	CI
Maternal Age	26.0	24.3	0.24	-1.2, 4.6
Race			0.97	
– Black	7 (13%)	5 (9.2%)		
– White	17 (31.5%)	11 (20.4%)		
– Other	8 (14.8%)	6 (11.1%)		
EGA@	32.8 wks	32.2 wks	0.49	-1.1, 2.3
Cervical Dilation	1.8 cm	2.3 cm	0.20	-1.2, 0.03
PROM*	3	2	0.97	

EGA = estimated gestational age at amniocentesis

PROM = premature rupture of membranes

CI = confidence interval

Table V
PCR & Clinical Outcomes

Outcome	PCR (+) (n=30)	PCR(-) (n=24)	P-Value	CI
TFATD	16.1 days	28.7 days	0.02	2.0-23.2
Gestational Age at Delivery	35.1 wks	36.2 wks	0.08	-1.2-4.6
Newborn Weight	2,621 g	3,041	0.02	59-781
Newborn Stay	8.2 days	5.9 days	0.49	-4.2-8.9
Newborn Sepsis	3	1	0.48	
Demise	1	0	0.40	

TFATD = time from amniocentesis to delivery

Table VI
IL-6 & Clinical Outcomes

Outcome	Elevated IL-6 (n = 9)	Normal IL-6 (n = 43)	p-Value	CI
TFATD	10.4 days	23.4 days	0.07	-1.3-27.3
Gestational Age at Delivery	31.2 wks	36.4 wks	0.0001	3.5-6.9
Newborn Weight	1,899 g	2,970 g	0.0001	669-1,474
Newborn Stay	25.1 days	4.5 days	0.0001	13.4-28.0
Newborn Sepsis	1	3	0.56	
Demise	1	0	0.17	

TFATD = time from amniocentesis to delivery

Table VII
Patient Samples with Elevated IL-6

Patient (number)	IL-6 (pg/mL)	PCR (bact)	PCR (myco)	EGA (wks)	WT (g)	TFATD (days)
M001	1,236	neg	neg	32.3	2,038	0
T006	4,670	pos	pos	29.6	1,315	18
T009	1,140	pos	neg	34.3	2,455	51
T024	6,660	pos	neg	29.6	1,703	0
T034	2,045	pos	neg	24.4	750	17
T050	5,450	pos	neg	33.1	2,335	0
T051	604	neg	neg	34.1	2,545	4
T052	>4,000	pos	neg	27.6	951	1
T055	2,572	neg	neg	35.6	2,996	1

EGA = estimated gestational age

WT = weight

TFATD = time from amniocentesis to delivery